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Amino acid composition, available lysine content and in vitro protein digestibility of selected tropical crop seeds

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Abstract. As the search for alternative sources of food to alleviate hunger continues, this study was undertaken to determine nitrogen and amino acid content, chemical score, protein digestibility corrected amino acid score, available lysine and in vitro digestibility of 8 lesser known, wild tropical seeds, gathered in Nigeria. Results were contrasted with a tropical soybean variety (*Glycine max*, TGX 1660-15F). The investigated seeds were *Millettia thonningii*, *Gliricidia sepium*, *Lonchocarpus sericeus*, *Albizia zygia*, *Daneillia ogea* and *Afzelia bella* from the family of Leguminosae, *Diospyros mespiliformis* (Ebenaceae) and *Entandrophragma angolense* (Meliaceae). The crude protein content, based on nitrogen determination, was found to be lower in the wild seeds compared to soybean, which was partly due to the relatively high content of non-protein nitrogen. With reference to amino acid requirement and digestibility in most seed samples, lysine, followed by sulphur amino acids and threonine, were the limiting amino acids. It was concluded, that these less familiar wild seed plants may be used as valuable food or feed complements. However, further investigation is necessary to elucidate potential toxic and antinutritional factors.

Key words: Amino acid composition, Available lysine, Nigeria, Nitrogen content, Protein digestibility corrected amino acid score, Tropical crop seeds

Introduction

Protein foods, particularly animal protein, have continued to be in short supply in Nigeria and most developing countries. This contributes to protein-energy malnutrition being the most important nutritional problem, especially in regions where diets are derived mainly from starchy root and tuber crops. With the rate of agricultural food production ever lagging behind population increase, available protein sources are in short supply, high prices not withstanding [1–4]. Future projections do not show much improvement and a situation has arisen wherein farm animals compete with man for the

Table 1. Characterization of selected tropical crop seeds

Genus and species	Family	Described[a]	Toxicity[c]	Use as food or feed[c]
Millettia thonningii	Leguminosae	yes	?	Ruminants
Gliricidia sepium	Leguminosae	yes	yes	Ruminants (leaves)
Lonchocarpus sericeus	Leguminosae	no	yes	?
Albizia zygia	Leguminosae	yes	no	Humans, Ruminants
Daneillia ogea	Leguminosae	yes	no	Humans
Diospyros mespiliformis	Ebenaceae	no	no	Humans
Afzelia bella	Leguminosae	yes	no	Humans, Ruminants
Entandrophragma angolense	Meliaceae	yes	?	?
Glycine max (1660–15F)[b]	Leguminosae	—	no	Humans

[a]Described by Keay [41] in 'Trees in Nigeria'.
[b]Soybean seed sample analyzed in comparison to the wild seed samples.
[c]Ezeagu et al. [33]

same food. To overcome this problem, a solution must be sought through a multidisciplinary approach. One approach is the exploitation of previously neglected and lesser known plants existing in the wild and natural bushes or forests [5–6].

Currently, there is an upsurge of worldwide interest in the search for development of new plant species with food potentials as complements to traditional crops or staples which are now in short supply. The exploitation of inexpensive alternative sources of protein for man and/or farm animals could measurably reduce malnutrition [7–8]. Wild and lesser known plants could contain useful amounts of nutrients as indicated by some research [1, 9–11]. However, prior to their utilization, more data is needed to indicate their food and feed potential and their risks related to harmful substances.

There is little information on the present uses of these selected species and the latter will vary with localities. However, cases of food (*Diospyros mespiliformis, Daneillia ogea, Afzelia bella, Albizia zygia*), fodder use (*Afzelia bella, Millettia thonningii, Gliricidia sepium*) and toxicity (*Gliricidia sepium, Lonchocarpus sericeus*) are known. Also fuel and timber production (*Entandrophragma angolense, Millettia thonningii*) and medicinal uses (*Diospyros mespiliformis, Millettia thonningii, Entandrophragma angolense*) have been reported [12–13]. Most of these species are presently being screened for agroforestry systems [14]. Therefore, this study was undertaken to determine the amino acid composition of 8 wild tropical plant seeds compared to a soybean grown in Nigeria.

Materials and methods

Collection of seed samples. Mature seed samples (35–50 kg) were harvested during dry period (October–January) from villages around the city of Ibadan with the help of the natives. Collections were taken from several plants to get a representative sample for the Ibadan region. The samples were identified at the Forestry Research Institute, Ibadan. Table 1 information includes a short characterization of the tropical crop seeds analyzed. The soybean sample (*Glycine max*, TGX 1660-15F) was provided by The International Institute of Tropical Agriculture, Ibadan, Nigeria, and was analyzed for purposes of comparison to the wild seed samples. The raw seeds were milled to flours in a Wiley mill (Rekord A, Gbr. Jehmlich GmbH, Nossen, Germany) to pass a 0.5 mm mesh sieve and were stored at 4 °C until analysis. Moisture content was determined by drying at 110 °C using an oven (T 6030, Heraeus Instruments, Hanau, Germany) until weight constancy (at least for 24 hours).

Nitrogen analysis. Nitrogen was determined by the standard micro-Kjeldahl method [15] using a digestion apparatus (Kjeldatherm System KT 40, Gerhardt Laboratory Instruments, Bonn, Germany) and a titration system (T110-TR160-TA10-TM120, Schott–Geräte GmbH, Hofheim, Germany). The crude protein content was calculated by multiplying % N by the factor 6.25.

Determination of available lysine. Available lysine (fluorodinitrobenzene-reactive lysine) was estimated. The method of Carpenter [16] modified by Booth [17] was used.

In vitro digestibility analysis. In vitro digestibility of the samples was assayed. The multienzyme technique of Hsu et al. [18] was utilized.

Amino acid analysis. Sample preparation was based on the recommendations made in the Report of the Joint FAO/WHO Expert Consultation [19–20]. To determine the amino acid composition, the seed samples were hydrolysed with 6 N HCl (2.5 mg nitrogen per 150 ml HCl; 24 hours) under reflux by a continuous flow of nitrogen, evaporated to dryness (40 °C), washed twice with distilled water to remove residual HCl and dried again. Norleucine served as an internal standard. Cysteine and methionine, which are destroyed during the acid hydrolysis, were converted to acid-stable derivatives (cysteinic acid and methioninesulfone, respectively) by performic acid oxidation [21]. The oxidized samples were then hydrolysed with 6 N HCl as described above. For tryptophan determination, alkaline hydrolysis was performed according to Rowan et al. [22] using 4.3 N NaOH in teflon containers which were flushed with nitrogen and placed in an oven (T 6030, Heraeus Instruments, Hanau,

Germany) for 24 hours maintained at 110 °C. 5-methyltryptophan was used as an internal standard. The hydrolyzed samples were stored at −18 °C in citrate buffer at pH 2.2 prior to analysis. Amino acids were analyzed by ion-exchange chromatography with post-column ninhydrin detection according to a modified procedure described by Moore et al. [23] using an HPLC system (System Gold, Beckman Instruments, Inc., San Ramon, USA).

The protein digestibility corrected amino acid score (PDCAAS) of indispensable amino acids was calculated according to the recommendations of the Joint FAO/WHO Expert Consultation based on amino acid requirement of the preschool child, a protein factor of 6.25, and the in vitro protein digestibility value that was determined. The following equation was used [19]

$$\text{PDCAAS (\%)} = \frac{\text{(amino acid content in food protein)} \times \text{(true digestibility)}}{\text{(amino acid content in 1985 FAO/WHO/UNU pattern for ages 2–5 years)}}$$

The determinations were done in duplicate. Descriptive statistics are presented in Tables 2–5. All chemicals were purchased from Sigma–Aldrich Chemie, Deisenhofen, Germany; Merck, Darmstadt, Germany; or Fluka Chemie, Buchs, Switzerland and were of analytical grade.

Results and discussion

The moisture, nitrogen and crude protein contents of the various plant seeds are shown in Table 2. Marked differences in nitrogen content occured among seed varieties. Crude protein contents, based on the conversion factor N ×6.25, ranged from 43.50 in *Gliricidia sepium* to 5.44 % in *Diospyros mespiliformis* (Table 2). In comparison to soybean and some commonly cultivated legumes such as cowpea, pigeon pea, and lima beans [24–26], the contents of crude protein were found to be relatively high in *Gliricidia sepium*, *Lonchocarpus sericeus* and *Albizia zygia*. However, the conversion factor N ×6.25 used for purposes of uniformity for calculation in Table 2 may be corrected to account only for the nitrogen found as protein amino acids [20, 27]. These correction factors and the corrected crude protein contents are also shown in Table 2 for the seed varieties analyzed. The correction factor of 1.09 for the soybean seed sample is found to be in close agreement with those reported in the literature [20]. For the seed samples studied, the correction factors were found to be in the range of 1.18–2.40. This is different than most of the plant foodstuffs, including wheat, rice or sunflower seeds, which are known to have correction factors between 1.00 and 1.18 [20].

Table 2. Nitrogen and crude protein contents of selected tropical crop seeds

Seed	Moisture content	Nitrogen content (N)	Crude protein content (N×6.25)	Crude protein content Corrected[a]	Nitrogen found as ammonia-N	Nitrogen found as amino acid-N	Correction factor (Cf) for crude protein[b]
	(% fresh weight)				(% of total N)		
Milletia thonningii	4.71	2.88	18.00	14.63	14.0	81.6	1.23
Gliricidia sepium	6.77	6.96	43.50	29.00	13.6	66.8	1.50
Lonchocarpus sericeus	5.49	4.48	28.00	18.54	10.2	66.4	1.51
Albizia zygia	7.80	5.29	33.06	13.78	16.1	41.6	2.40
Daniella ogea	9.86	2.16	13.50	9.64	15.1	71.6	1.40
Diospyros mespiliformis	8.99	0.87	5.44	4.32	16.9	79.1	1.26
Afzelia bella	6.81	2.09	13.06	10.70	13.3	82.3	1.22
Eriandrophragma angolense	2.63	1.97	12.31	10.43	17.0	85.0	1.18
Glycine max	6.61	6.19	38.69	35.50	14.5	91.9	1.09

[a]Calculated according to the formula: (N×6.25)/(Cf).
[b]Correction factor for crude protein based on nitrogen content of proteinogenic amino acid origin. (Cf) = 100/(% Nitrogen as amino acids).

The legumes are generally high in protein and, thus, have the greatest promise as a concentrated source of low cost plant protein [25]. High crude protein levels have also been reported in some lesser known tropical legumes [28–29]. However, protein content of legumes may differ widely, with as much variation within species as among them [30]. After correction for protein nitrogen, however, levels are relatively low in the seed samples studied and only *Gliricidia sepium* (29.00%) was found to be relatively similar to the soybean sample (35.50%) regarding the protein content (Table 2).

The percentage of nitrogen determined as ammonia ranged between 10 and 17% (Table 2) and may be, in part, due to amides of glutamic acid and aspartic acid and unstable non-proteinogenic amino acids known to be present in seeds of several plant species [31]. The nitrogen originating from proteinogenic amino acids also shows a wide variation among different seed varieties and ranges from 41.6% for *Albizia zygia* to 85.0% for *Entandrophragma angolense*. In comparison, the *Glycine max* seed sample had a value of 91.9% for proteinogenic amino acid nitrogen. This variation has been confirmed for various species of Leguminosae seeds [31]. A high percentage of nitrogen remained unidentified in *Daneillia ogea* (13.3%), *Gliricidia sepium* (19.6%), *Lonchocarpus sericeus* (23.4%) and *Albizia zygia* (42.6%). This may be, in part, due to other amino acids of non-protein origin such as α,β-diaminopropionic acid or canavanine. Canavanine was found in significant amounts in several seeds from the Leguminosae family [31]. In non-oxidized samples of *Gliricidia sepium* and *Albizia zygia*, several non-identified compounds (20 and 15% of total area, respectively) eluting between histidine and lysine have been observed.

The amino acid composition of the seeds investigated is based on total nitrogen (Table 3). To our knowledge, this is the first report on the amino acid composition of these tropical seed samples with the exception of *Glycine max*. Table 4 contains a summary of the PDCAAS-values. A high content of sulphur amino acids was determined in seeds of *Entandrophragma angolense*, *Daneillia ogea* (cysteine) and *Diospyros mespiliformis* (cysteine and methionine).

The in vitro protein digestibility of the raw seed samples ranged from 56 to 75% (Table 4). These results for raw seeds are similar to those reported for legumes ranging from 70 to 78% [32] except for *Diospyros mespiliformis* (56%, Table 4) which belongs to the Ebenaceae family (Table 1). Antinutritional factors present in leguminous and other seeds may lower the digestibility of proteins in vivo and in vitro. Among them, the heat-unstable components such as protease inhibitors and lectins or the heat-stable factors phytate and tannins are the most important seed constituents which may modify the availability of amino acids [30]. Furthermore, the carbohydrate

Table 3. Amino acid composition of selected tropical crop seeds analysed by ion-exchange chromatogrphy

Seed	Aspartic acid	Threonine	Serine	Glutammic acid	Proline	Glycine	Alanine	Valine	Isoleucine
	(mg/gN)								
Millettia thonningii	606	221	300	842	281	323	246	267	219
Gliricidia sepium	427	138	199	1068	205	221	210	206	182
Lonchocarpus sericeus	561	202	259	719	257	226	210	236	204
Albizia zygia	298	108	174	658	179	160	144	149	132
Daneillia ogea	549	266	287	691	321	382	219	298	266
Diospyros mespiliformis	507	231	291	1002	324	336	295	287	228
Afzelia bella	625	249	277	897	371	420	287	321	279
Entandrophragma angolense	284	110	237	1858	382	271	168	268	269
Glycine max	778	256	336	1249	426	293	277	317	293

Seed	Leucine	Tyrosine	Phenylalanine	Histidine	Lysine	Arginine	Cysteine	Methionine	Tryptophan
	(mg/gN)								
Millettia thonningii	467	210	306	118	330	631	111	51	85
Gliricidia sepium	359	152	219	130	248	504	81	51	66
Lonchocarpus sericeus	441	207	307	117	375	337	91	37	58
Albizia zygia	242	131	137	85	219	174	38	17	46
Daneillia ogea	419	280	222	107	340	283	237	92	82
Diospyros mespiliformis	398	191	243	126	301	501	151	113	92
Afzelia bella	472	306	253	138	317	441	137	76	93
Entandrophragma angolense	384	172	227	164	283	532	570	40	75
Glycine max	507	265	334	170	422	499	144	69	100

Table 4. Protein digestibility corrected amino acid score (PDCAAS) of indispensable amino acids (including histidine) and in vitro protein digestibility (IVPD) of selected tropical crop seeds (in %)

Seed	Threonine	Valine	Isoleucine	Leucine	Tyrosine +Phenyla-lanine	Histidine	Lysine	Cysteine +Metbio-nine	Trypto-phan	IVPD
Millettia thonningii	71	83	85	77	89	67	62*	71	85	68
Gliricidia sepium	46*	66	73	61	66	76	48	59	67	70
Lonchocarpus sericeus	64	72	78	71	88	66	69	55*	57	67
Albizia zygia	36	48	54	42	48	51	43	25*	47	71
Daniella ogea	87	94	105	71	88	62*	65	146	82	69
Diospyros mespiliformis	61	73	73	54	62	60	47*	95	75	56
Afzelia bella	84	106	114	82	102	83	63*	98	97	72
Entandrophragma angolense	37*	87	109	66	72	98	56	256	77	71
Glycine max	90	109	125	92	114	108	87*	102	109	75

*Indicating the value of the first limiting amino acid.
PDCAAS: Based on amino acid requirement of the preschool child, protein factor 6.25 and in vitro protein digestibility.

Table 5. Available lysine content of selected tropical crop seeds

Seed	Available lysine (mg lysine/g N)	Available lysine as % of total lysine (%)
Millettia thonningii	378[a]	115[a]
Gliricidia sepium	204	82
Lonchocarpus sericeus	243	65
Albizia zygia	511[a]	233[a]
Daneillia ogea	284	84
Diospyros mespiliformis	424[a]	141[a]
Afzelia bella	282	89
Entandrophragma angolense	273	96
Glycine max	331	78

[a]Values overestimated as proposed to be due to sample matrix.

moieties in some glycoproteins found in leguminous proteins may contribute to a partial resistance to proteolytic digestion. Seeds from *Diospyros mespiliformis* were determined to contain a high percentage of total carbohydrates (77.2% [33]), thus, making the presence of such glycoproteins in some of the seeds possible.

Based on the human amino acid requirements [19] and in vitro protein digestibility values (Table 4), in most seed samples, lysine was calculated to be the first or second limiting amino acid followed by the sulphur amino acids (*Lonchocarpus sericeus, Albizia zygia*) and by threonine (*Gliricidia sepium, Entandrophragma angolense*) (Table 4). Calculation of the protein digestibility corrected amino acid scores is usually performed using the true digestibility values. However, it has been shown that the in vitro protein digestibility compares favorably with those obtained by in vivo methods applied in rats [34–36]. Furthermore, it should be noted that the low amino acid score values for all indispensable amino acids of the samples *Albizia zygia, Gliricidia sepium* or *Lonchocarpus sericeus* (Table 4) may be attributed to the low ratio of proteinogenic amino acid nitrogen to total nitrogen as discussed above.

Fluorodinitrobenzene-reactive lysine seems not to be a feasible indicator for nutritionally available lysine in some tropical seeds in contrast to its value in evaluating oilseeds [37] or rice bran [38]. In this study, artificially high values were observed in seeds of *Millettia thonningii, Diospyros mespiliformis* and *Albizia zygia* (Table 5) which may be due to sample matrix factors related to the carbohydrate constituents [17, 20]. For the remaining seed samples, the available lysine expressed in percent relative to total lysine determined by

amino acid analysis ranged between 65 % (*Lonchocarpus sericeus*) and 96% (*Entandrophragma angolense*).

Considering the data presented herein, some of these tropical crop seeds could play a substantial role in alleviating food and feed shortages in Nigeria and, elsewhere, if given adequate attention [9, 39–40]. These seeds may possibly be utilized in supplementing diets or feeds for cattle or poultry and, thus, spare more of the scarce staples for human consumption. The low levels of particular indispensable amino acids could be corrected by fortifying the seed meals with appropriate purified amino acids [27]. However, further research into possible toxic constituents and their removal or inactivation through proper processing would be necessary.

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